

# Detection of Methicillin-Resistant Coagulase-Negative Staphylococci by the Vitek 2 System

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The accurate performance of the Vitek 2 GP67 card for detecting methicillin-resistant coagulase-negative staphylococci (CoNS) is not known. We prospectively determined the ability of the Vitek 2 GP67 card to accurately detect methicillin-resistant CoNS, with *mecA* PCR results used as the gold standard for a 4-month period in 2012. Included in the study were 240 consecutively collected nonduplicate CoNS isolates. Cefoxitin susceptibility by disk diffusion testing was determined for all isolates. We found that the three tested systems, Vitek 2 oxacillin and cefoxitin testing and cefoxitin disk susceptibility testing, lacked specificity and, in some cases, sensitivity for detecting methicillin resistance. The Vitek 2 oxacillin and cefoxitin tests had very major error rates of 4% and 8%, respectively, and major error rates of 38% and 26%, respectively. Disk cefoxitin testing gave the best performance, with very major and major error rates of 2% and 24%, respectively. The test performances were species dependent, with the greatest errors found for *Staphylococcus saprophyticus*. While the 2014 CLSI guidelines recommend reporting isolates that test resistant by the oxacillin MIC or cefoxitin disk test as oxacillin resistant, following such guidelines produces erroneous results, depending on the test method and bacterial species tested. Vitek 2 cefoxitin testing is not an adequate substitute for cefoxitin disk testing. For critical-source isolates, *mecA* PCR, rather than Vitek 2 or cefoxitin disk testing, is required for optimal antimicrobial therapy.

"he detection of methicillin resistance (MR) in coagulase-negative staphylococci (CoNS) can be critically important for isolates from normally sterile sites. However, detection of MR CoNS is problematic and less reliable than the detection of MR Staphylococcus aureus (1, 2). Cefoxitin susceptibility testing has greatly improved the reliability of detecting MR S. aureus and, to a lesser extent, CoNS (3, 4). Current CLSI guidelines recommend the use of cefoxitin disk testing for detecting MR CoNS, with some evidence that cefoxitin MIC determination can serve the same purpose (3, 5). A number of studies have shown that cefoxitin disk testing of CoNS is generally sensitive but can be nonspecific (3, 4, 6, 7). The Vitek 2 (Vitek) antimicrobial susceptibility system utilizes a cefoxitin susceptibility screening assay that was previously reported to have a 98% sensitivity for MR and a 100% specificity for S. epidermidis but only 66% and 100% sensitivity and specificity, respectively, for S. hominis (6). This led our laboratory to perform cefoxitin disk testing for S. hominis isolates rather than rely on the Vitek cefoxitin test. In addition, when the Vitek oxacillin result was discordant from the Vitek cefoxitin result, cefoxitin disk testing was performed. We found, however, that there were a large number of discrepancies between the Vitek cefoxitin and disk cefoxitin tests. Over the period from January to December 2011, we encountered 25 Vitek cefoxitin-susceptible CoNS isolates that were cefoxitin disk resistant, as well as 47 Vitek cefoxitin-resistant CoNS isolates that were cefoxitin disk susceptible; 72% and 6% of those two groups, respectively, were positive by mecA PCR. In order to determine a more accurate estimate of the cefoxitin test discordant incidence and to better understand the reasons for such discordant results, we performed a 4-month prospective study of all CoNS that had undergone susceptibility testing. We did this to determine if the Vitek cefoxitin screening test was reliable in our laboratory and, if not, whether alternative phenotypic methods were more reliable. The results from this

prospective study are given here. We show that the Vitek cefoxitin screening test is both insensitive and nonspecific.

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## **MATERIALS AND METHODS**

Consecutive nonduplicate clinical isolates of CoNS that were tested for drug susceptibility using the Vitek (bioMérieux, Durham, NC) were collected from September to December 2012. A grand total of 240 isolates were collected that had been grown from blood, urine, and wound specimens. All isolates were identified to the species level using the Vitek GP card and, in the case of ambiguous identification, by 16S rRNA gene sequencing (8). The isolates were subcultured twice onto tryptic soy 5% sheep blood agar plates (BBL Trypticase soy agar [TSA] II; BD Diagnostic Systems) in ambient air at 35°C before testing.

Cefoxitin screening for oxacillin resistance and susceptibility of the CoNS isolates was determined using the Vitek GP67 card; oxacillin and cefoxitin testing procedures of all staphylococci, except for cefoxitin testing of *S. saprophyticus*, are listed as indications in the product insert (9). The oxacillin MICs were interpreted according to the staphylococcal species-specific CLSI breakpoints (5): susceptible,  $\leq 0.25 \mu g/ml$ , and resistant,  $\geq 0.5 \mu g/ml$  for CoNS other than *S. lugdunensis*; susceptible,  $\leq 2.0 \mu g/ml$ , and resistant,  $\geq 4.0 \mu g/ml$  for *S. lugdunensis*. The

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TABLE 1 Sensitivities and specificities of Vitek 2 and cefoxitin disk susceptibility testing for detecting *mecA*-positive coagulase-negative staphylococci

Organism (no. of isolates)	% mecA positive	Performance (%) of <sup>a</sup> :								
		Vitek oxacillin		Vitek cefoxitin		Vitek oxacillin and cefoxitin		Disk cefoxitin		
		Sens	Spec	Sens	Spec	Sens	Spec	Sens	Spec	
S. epidermidis (140)	66	97	90	93	90	97	88	98	88	
S. saprophyticus (41)	12	100	3	100	39	100	3	100	56	
S. haemolyticus (23)	43	100	92	100	100	100	92	100	85	
S. hominis (11)	55	66	80	66	100	83	80	100	80	
S. lugdunensis (8)	0		88		88		88		88	
S. capitis (4)	25	100	67	100	100	100	67	100	100	
S. warneri (4)	25	100	100	100	67	100	67	100	100	
S. simulans (4)	25	100	100	0	100	100	100	100	100	
S. auricularis (2)	0		0		0		0		0	
S. lentus (1)	100	100		100		100		100		
S. caprae (1)	0		100		100		100		100	
S. xylosus (1)	0		0		0		0		0	
Total (240)	49	96	62	92	74	97	60	98	76	

 $<sup>^</sup>a$  Sens, sensitivity; Spec, specificity.

Vitek interpretive software uses the species identification to determine the appropriate oxacillin breakpoint that should be used in each case, and it warns that oxacillin susceptibility testing performed without species identification may result in the use of the wrong breakpoint. The Vitek reports only qualitative values for cefoxitin, as either a positive or a negative result for MR, as determined by the susceptibility of a test isolate to 6  $\mu$ g/ml cefoxitin.

Cefoxitin disk diffusion testing was performed using 30-µg cefoxitin disks (BD-BBL Sensi-Disc) and Mueller-Hinton agar (Remel) and interpreted as specified by CLSI guidelines (5). The CLSI-specified resistance zone diameter was  $\leq\!24$  mm for CoNS other than S. lugdunensis and  $\leq\!21$  mm for S. lugdunensis. Agar dilution susceptibility testing was performed in duplicate to determine the oxacillin MICs of 52 CoNS isolates with discrepant Vitek oxacillin/mecA test results, according to CLSI guidelines. Laboratory-prepared Mueller-Hinton agar (Difco) supplemented with 2% NaCl was inoculated with the bacteria using a Steers-Foltz Graves replicator (10). The oxacillin MICs were interpreted according to CLSI guidelines (5). The geometric mean MICs were reported when the duplicate values disagreed.

PCR detection of mecA was performed as described previously (6, 11), with slight modifications. Briefly, bacterial colonies were suspended in 100 µl of sterile distilled water and heated in a heating block at 100°C for 10 min. The supernatant (1 μl) was used in a 25-μl PCR assay containing 200 nM each primer and illustra Hot Start Mix RTG (GE Healthcare Life Sciences, Pittsburgh, PA). The PCR cycling conditions were 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. Both an MJ PTC-200 and an MJ Mini personal thermal cycler (Bio-Rad) were used for PCR. The presence of the expected size (500 bp) PCR product was determined by gel electrophoresis using E-Gel 2% with SYBR safe (Life Technologies); only PCRs yielding strong single-band products were scored as positive for mecA. Both positive- and negative-control bacterial lysates were included in each PCR run. If the lysate PCR yielded only a faint band of the correct size, purified bacterial DNA (QIAamp DNA minikit; Qiagen) was used in a repeat PCR test to exclude the presence of PCR inhibitors.

Statistical analysis of the matched case-control categorical data was performed using the McNemar test (QuickCalcs; GraphPad). The calculation of 95% confidence intervals of a proportion was performed using the same software.

#### **RESULTS**

The 240 bacterial isolates were identified as belonging to 12 different species of CoNS (Table 1). The most common species included *S. epidermidis* (59%), *S. saprophyticus* (17%), *S. haemolyticus* (10%), and *S. hominis* (5%). PCR testing for *mecA* was positive in 117 of the 240 (49%) isolates. *S. epidermidis* comprised 78% (92/117) of the *mecA*-positive isolates. The remaining *mecA*-positive *Staphylococcus* spp. were *S. haemolyticus* (8.5% [10/117]), *S. hominis* (5.1% [6/117]), *S. saprophyticus* (4.3% [5/117]), and four additional species that comprised <1% (1/117) each.

The performances of Vitek and cefoxitin disk testing for the detection of MR CoNS are shown in Table 1 and also in Table S1 in the supplemental material. The Vitek oxacillin testing and cefoxitin disk testing detected 96% and 98%, respectively, of the MR isolates. In contrast, the Vitek cefoxitin screening test was significantly less sensitive for detecting MR than was cefoxitin disk testing (92% versus 98%, P = 0.02 by McNemar test). The test specificity was poor for both of the Vitek methods and the cefoxitin disk test, ranging from 62% to 76%; the cefoxitin-based tests were the most specific overall. When the combined Vitek oxacillin and cefoxitin test results were used to determine oxacillin resistance, the test performance did not change significantly.

To determine the accuracy of the Vitek oxacillin test, the oxacillin agar dilution MICs were determined for those isolates with discrepant mecA PCR and Vitek oxacillin MIC results (Table 2). Of the five mecA-positive but Vitek oxacillin-susceptible isolates, two were oxacillin resistant by agar dilution; the oxacillin MICs of the remaining three isolates were 0.06, 0.18, and 0.35  $\mu$ g/ml. Of the 47 mecA-negative but Vitek oxacillin-resistant isolates, 35 of which were from S. saprophyticus, 13 isolates were oxacillin susceptible by agar dilution; 30 of the 34 isolates that were oxacillin resistant by both methods were from S. saprophyticus. The oxacillin agar MICs of the 34 oxacillin-resistant but mecA-negative isolates ranged from 0.5 to >8.0 (median, 0.5)  $\mu$ g/ml. Of the 39 isolates with Vitek oxacillin MICs between 0.5 and 2  $\mu$ g/ml, all had

TABLE 2 Oxacillin susceptibility by agar dilution for coagulase-negative staphylococci with discrepant Vitek and *mecA* PCR results

	No. of oxacillin-susceptible organisms detected by <sup>a</sup> :						
mecA status	Vitek		Agar dilution				
	R	S	R	S			
+		5 <sup>b</sup>	2	3 <sup>c</sup>			
_	$47^{d}$		$34^e$	13			

- <sup>a</sup> R, resistant; S, susceptible.
- <sup>b</sup> S. epidermidis (3) and S. hominis (2).
- <sup>c</sup> S. epidermidis(2) and S. hominis (1).
- <sup>d</sup> S. saprophyticus (35), S. epidermidis (5), S. hominis (1), S. haemolyticus (1), and others (5).
- <sup>e</sup> S. epidermidis (2), S. saprophyticus (30), and others (2).

oxacillin agar MICs of <2 µg/ml, with 30 of 39 isolates having oxacillin agar MICs between 0.5 and 2 µg/ml.

For CoNS isolates other than *S. epidermidis* that cause serious infections and have intermediate-range oxacillin MICs (0.5 to 2.0 µg/ml), CLSI guidelines recommend testing the isolate for *mecA*, penicillin-binding protein 2A (PBP2A), or for cefoxitin susceptibility by disk diffusion (5). Cefoxitin disk diffusion testing incorrectly identified 41% (17/41) of the CoNS isolates from all sites with intermediate oxacillin susceptibility as being MR; 54% of the isolates were both cefoxitin disk negative and *mecA* negative, with 5% of the isolates being both *mecA* positive and cefoxitin disk positive. Fifteen of the 17 isolates that were falsely determined to be cefoxitin disk resistant were from *S. saprophyticus*. Only three of the 41 CoNS isolates tested were isolated from blood cultures, with the remainder being isolated from urine specimens. Of the three potentially invasive isolates, one isolate was falsely classified as being MR by the cefoxitin disk test.

Significant differences in test performance were observed, depending on the staphylococcal species tested. Therefore, we recalculated test performance after excluding results from the species associated with poorer test performance. Because Vitek oxacillin test sensitivity was lowest for S. hominis, the overall Vitek oxacillin test performance was recalculated without including this species; this had a negligible effect on overall test sensitivity, changing it from 96% to 97%, with no effect on specificity. The performance of the Vitek cefoxitin screening test was recalculated after excluding *S. saprophyticus*; this increased the overall test specificity from 74% to 89%, without changing test sensitivity. The exclusion of *S*. saprophyticus from the performance calculation of the combined Vitek test for MR CoNS had no effect on test sensitivity but increased test specificity from 60% to 84%. By omitting S. saprophyticus from the calculation of the test performance of the cefoxitin disk diffusion test, the overall specificity increased from 76% to 85%.

### **DISCUSSION**

Our study shows that the Vitek cefoxitin screen has relatively poor performance, with neither its sensitivity nor its specificity meeting the FDA guidelines for test performance (12). The FDA guidelines recommend lower and upper bounds of the 95% confidence interval (CI) of  $\leq$ 1.5% and  $\leq$ 7.5%, respectively, for very major errors, and an average value of  $\leq$ 3% for major errors, as opposed to the 8% (95% CI, 3.6% to 14%) and 26% values, respectively, found in our study. John et al. (6) reported the overall sensitivity and specificity of the Vitek cefoxitin test to be 88% and 91%,

respectively, in contrast to our findings of 92% and 74%, respectively (6). The performance of the Vitek cefoxitin screen and the cefoxitin disk test can be improved simply by not testing *S. saprophyticus*, which is associated with low-specificity MR detection (6, 7, 13), or by performing an alternative test method on *S. saprophyticus* isolates with positive cefoxitin screen results (9). By eliminating *S. saprophyticus* from our test results, the Vitek cefoxitin test specificity increased from 74% to 89%. However, even with this adjustment, neither the test sensitivity nor specificity improved enough to meet the FDA performance guidelines.

The Vitek oxacillin MIC test for the determination of MR CoNS was more sensitive at 96% than the cefoxitin screen test at 92%, but it fell just outside (95% CI, 1.6% to 9.8%) the FDA-specified criteria. However, the specificity of this test was poor at 62%, falling well outside FDA guidelines. The test specificity, but not sensitivity, was significantly enhanced by excluding *S. saprophyticus* from testing, increasing the test specificity from 62% to 87%. Just as with the Vitek cefoxitin testing, the exclusion of *S saprophyticus* from testing did not improve test performance enough to meet the FDA guidelines.

Determining how to practically resolve these test performance issues is not straightforward. While the CLSI recommends testing non-S. epidermidis species with intermediate MICs by cefoxitin disk testing, mecA PCR, or MecA protein detection, we found that the cefoxitin disk performance was not always optimal. Cefoxitin disk testing of non-S. epidermidis isolates with intermediate oxacillin MICs is a reliable measure of MR but overcalls resistance for S. saprophyticus and S. haemolyticus. If S. saprophyticus is excluded from testing, the Vitek oxacillin MIC was reliable enough to determine when to perform cefoxitin disk testing. The high major error rates of the phenotypic tests mean that in some cases, the molecular detection of mecA may be indicated to avoid the unneeded use of a non-\u00a8-lactam drug. If a laboratory is unable to identify all coagulase-negative staphylococci to the species level, test performance may be suboptimal for aiding in the interpretation of oxacillin/cefoxitin susceptibility testing. In such a case, the use of screening assays to detect *S. hominis*, *S. lugdunensis*, and *S.* saprophyticus might be used to tailor the MR detection method that is appropriate for the staphylococcal species to be tested.

One possible shortcoming of this study is that *mecA*-negative isolates were not tested for the presence of *mecC*. However, *mecC* is a very rare cause of MR *S. aureus* globally (14) and an apparently rarer cause of MR in CoNS (15). Therefore, it is very unlikely that not testing for *mecC* affects our conclusions.

Based on our study results, we changed our testing protocols. We no longer perform antimicrobial susceptibility testing of S. saprophyticus; matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is used to identify these isolates before susceptibility testing is performed. Prior to the use of MALDI-TOF MS, we set up a novobiocin disk test at the same time that susceptibility testing was done and did not report the susceptibility results if the isolate was novobiocin resistant, despite the imperfect performance of novobiocin identification of S. saprophyticus (16, 17). The Vitek 2 GP identification card is accurate for identifying most coagulase-negative staphylococci, including the Staphylococcus spp. that give the biggest errors in oxacillin susceptibility testing, so this system alone can be used to identify the problem species (18–21). We continue to test S. hominis using a cefoxitin disk test, regardless of Vitek results for MR, relying exclusively on the disk test results. We use mecA PCR on

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critical-site (normally sterile) *S. epidermidis* isolates that are reported as methicillin susceptible by Vitek. Finally, *mecA* PCR is conducted on other critical site isolates of non-*S. epidermidis* CoNS with oxacillin MICs in the intermediate range. For CoNS isolates from urine samples sites with intermediate-range oxacillin MICs, we use the cefoxitin disk test for all species except *S. saprophyticus*. If oxacillin susceptibility testing is required for *S. saprophyticus*, the only reliable method appears to be detection of *mecA* by PCR or MecA production. These protocols are not perfect, because we will continue to report falsely MR CoNS results, but performing *mecA* PCR on all CoNS isolates requiring susceptibility testing is impractical for our laboratory.

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